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RESEARCH ARTICLE

Two *C*-Methyl Derivatives of [^{11}C]WAY-100635–Effects of an Amido α-Methyl Group on Metabolism and Brain 5-HT$_{1A}$ Receptor Radioligand Behavior in Monkey*

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Abstract

Purpose: [*carbonyl*-^{11}C]*N*-(2-(1-(4-(2-methoxyphenyl)-piperazinyl)ethyl)-*N*-pyridinyl)cyclohexanecarboxamide ([*carbonyl*-^{11}C]WAY-100635) is an effective radioligand for imaging brain 5-HT$_{1A}$ receptors with positron emission tomography (PET). However, this radioligand has some drawbacks for deriving relative regional receptor densities, including rapid metabolism, which acts against accurate definition of an arterial input function for compartmental modeling, and very low nonspecific binding in brain, which detracts from the accuracy of modeling by a simplified reference tissue (cerebellum) approach. Here, in a search for a radioligand that overcomes these limitations, we investigated the effects of introducing a single methyl group at either of the carbon atoms alpha to the amide bond in [^{11}C]WAY-100635.

Procedures: Ligands with a methyl group on the alpha carbon of the cyclohexyl group (SWAY) or the alpha carbon of the C_2H_4 linker ((*R*,*S*)-JWAY) were synthesized and tested for binding affinity and intrinsic activity at 5-HT$_{1A}$ receptors. SWAY was labeled with carbon-11 ($t_{1/2}$ = 20.4 minutes; β^+ = 99.8%) in its *O-methyl* group and (*R*,*S*)-JWAY in its carbonyl group. Each radioligand was evaluated by PET experiments in cynomolgus monkey.

Results: SWAY and (*R*,*S*)-JWAY were found to be high-affinity antagonists at 5-HT$_{1A}$ receptors. After injection of [^{11}C]SWAY into monkey, radioactivity uptake in brain reached a maximum of 3% at 4.5 minutes and decreased to 0.7% at 72 minutes. However, over the time span of the experiment, radioactivity concentrations in 5-HT$_{1A}$ receptor-rich brain regions were only fractionally higher than in cerebellum. Radioactivity represented by parent radioligand in plasma

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was 39% at 45 minutes. After injection of [^{11}C](*R,S*)-JWAY alone, radioactivity uptake in brain reached a maximum of 4.8% at 2.5 minutes and decreased to 1.2% at 90 minutes. At this time, radioactivity concentration in 5-HT$_{1A}$ receptor-rich brain regions was markedly greater than in cerebellum. In another PET experiment, the monkey was predosed with WAY-100635 before [^{11}C](*R,S*)-JWAY injection. At 90 minutes after injection, the ratio of radioactivity in 5-HT$_{1A}$ receptor-rich regions to that in cerebellum was reduced to near unity. Radioactivity represented by parent radioligand in plasma was 12% at 45 minutes.

Conclusions: [^{11}C](*R,S*)-JWAY, but not [^{11}C]SWAY, gives a sizeable 5-HT$_{1A}$ receptor-selective PET signal in monkey. The presence of a *C*-methyl group adjacent to the amide bond in SWAY or (*R,S*)-JWAY fails to counter metabolism.

Key words: 5-HT$_{1A}$ receptors, PET, Radioligand, WAY-100635, α-Methyl, Metabolism, Carbon-11

Introduction

WAY-100635 (**1** in Fig. 1), labeled in its carbonyl group with carbon-11 ($t_{1/2}$ = 20.4 minutes; β^+ = 99.8%) [1], has gained widespread importance with positron emission tomography (PET) for assessing the role of brain 5-HT$_{1A}$ receptors in neuropsychiatric disorders and for assessing 5-HT$_{1A}$ receptor occupancy by therapeutics and developmental drugs [2, 3]. Estimates of binding potential in brain 5-HT$_{1A}$ receptor-rich regions can be made by applying biomathematical models to acquired PET data [4–6]. However, the rapid metabolism of this radioligand by amide hydrolysis [7] in human subjects causes some difficulty in applying radioligand kinetic models that require an arterial input function [8]. Alternative reference tissue models also have a drawback due to the low nonspecific binding of the radioligand (low radioactivity) in the reference region (cerebellum) [9, 10].

A structural analogue of WAY-100635 known as CPC-222 (**2**), where the cyclohexyl group has been replaced by the bulkier 2-bicyclo[2,2,2]octanyl group, has been labeled with carbon-11 and shown to resist amide hydrolysis in human liver microsomes *in vitro* [11, 12] and in human subjects [12, 13]. CPC-222 is an antagonist like WAY-100635 [14]. However, the 2-bicyclo[2,2,2]octanyl group of CPC-222, relative to the cyclohexyl group of WAY-100635, adds some lipophilicity and slightly reduces affinity (Table 1), which are each deleterious to obtaining high receptor-selective PET signals in brain. Hence, in human brain, [*O-methyl*-^{11}C]CPC-222 gives a smaller 5-HT$_{1A}$ receptor-specific signal [11, 12] than [*carbonyl*-^{11}C]WAY-100635 [1].[1]

Since analogs of WAY-100635 bearing bulkier cycloalkylcarbonyl groups, such as CPC-222, appear to be more resistant to metabolism by amide hydrolysis [11–13], we were interested to see if a similar effect might be achieved by simply adding a smaller group, specifically a methyl group, to WAY-100635 for expectedly less impact on lipophilicity and pharmacology. In this study, we explored the effects on pharmacology, metabolism, and radioligand behavior in monkey of placing a methyl group at either carbon atom alpha to the amido group in WAY-100635. The two ligands from this approach are SWAY (**3**) (α-methyl group in the cyclohexyl ring) and (*R,S*)-JWAY (**4**) (α-methyl group in the side chain).

Methods

Materials

2-Methoxyphenyl piperazine (**7**) and α-methylcyclohexanecarbonyl chloride were obtained from Aldrich. 1-(2-Methoxyphenyl)-4-(2-(2-aminopyridinyl)ethyl)-piperazine (WAY-100634) (**5**) was prepared from **7** as described previously [16]. All other chemicals were purchased and of analytical grade. Columns (μ-Bondapak-C18) for high-performance liquid chromatography (HPLC) were purchased from Waters Associates.

General Methods

^{1}H NMR spectra were obtained at 300 K in $CDCl_3$ on Varian Gemini (300 or 200 MHz) instruments or in CD_3CN on a Bruker

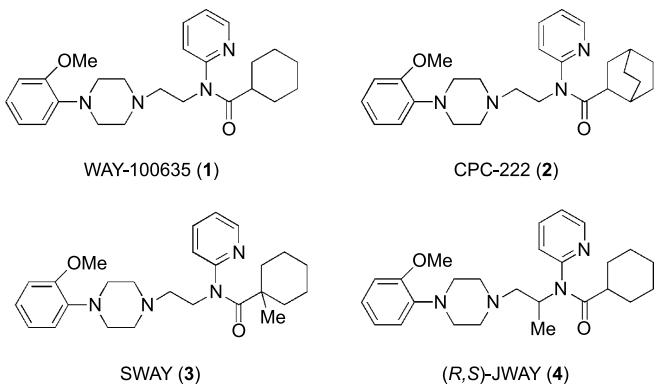

Fig. 1. Structures of 5-HT$_{1A}$ receptor ligands.

[1]The size of the signal from [*O-methyl*-^{11}C]CPC-222 is, however, similar to that from the similarly labeled [*O-methyl*-^{11}C]WAY-100635 [1].

Table 1. Properties of 5-HT$_{1A}$ receptor antagonists

Ligand	Affinity		Lipophilicity	
	K_i (nM)	IC_{50} (nM)	LogP	LogD
WAY-100635 (**1**)	0.37	2.2	3.28	2.88
CPC-222 (**2**)	ND[a]	4.2	3.60	2.87
SWAY (**3**)	2.3	ND[a]	3.42	2.69
(*R*,*S*)-JWAY (**4**)	0.91	2.3[b] 1.75[c]	3.81	3.08

[a] Not determined or reported.
[b] R-enantiomer [24, 25].
[c] S-enantiomer [24, 25].

AM250 (200.13 MHz) instrument. ^{13}C NMR spectra were obtained at 300 K in $CDCl_3$ on a Varian Gemini (50 MHz) instrument or in CD_3CN on a Bruker AM250 (62.9 MHz) instrument. Chemical shifts are reported in δ values (ppm), by reference to the hydrogenated residues of deuteriated solvent as internal standard. Signals are described as s, d, t, dd, dt, and m for singlet, doublet, triplet, double doublet, double triplet, and multiplet, respectively. Where shown, ^{13}C NMR signals were assigned to C, CH, CH_2, and CH_3 carbons with distorsionless enhancement by polarization transfer (DEPT) analysis.

Infrared (IR) analysis was performed on an ATI-Mattson spectrometer or Spectrum 1 FT-IR spectrometer (Perkin-Elmer).

Melting points are uncorrected and were determined with an Electrothermal apparatus (MEL-TEMP®).

Mass spectra [electrospray (ES)] were recorded on a Sciex API 3000 instrument or on a Unicam 610/Automass 150 gas chromatography–mass spectrometry (GC–MS) system [electron ionization (EI)]. High-resolution mass spectrometry (HRMS) data were obtained by the Microanalytical Department of the University of Groningen on a Jeol MS Route JMS600H instrument.

Analytical thin layer chromatography (TLC) was performed on silica gel layers (60 PF 254 precoated aluminum sheets, 0.2-mm layer; Merck).

[^{11}C]Carbon dioxide was produced batchwise from the ^{14}N(p,α)^{11}C reaction with the Scanditronix MC16 cyclotron at the Karolinksa Institutet to bombard a nitrogen gas target with 16 MeV protons.

The radiochemical purities and specific radioactivities of radioligands were measured by high-performance liquid chromatography (HPLC) on a μ-Bondapak-C18 column (10-μm particle size; 30 × 0.39 cm) eluted with MeCN–0.01 M phosphoric acid (35:65 v/v) at 3.0 ml/min, with eluate monitored sequentially for absorbance at 270 nm and radiation (Beckman β-flow detector). Radioligands were coinjected with reference ligands on the same HPLC system to verify identity.

Lipophilicity and Pharmacology

LogP and logD values[2] for ligands **1**–**4** were computed from drawn molecular structures using the program Pallas 3.0 for Windows. Binding affinities were determined as K_i values for the inhibition of binding of [^{3}H]5-carboxamidotryptamine ([^{3}H]CT) to human cloned 5-HT$_{1A}$ receptors *in vitro* [17]. Intrinsic efficacies at 5-HT$_{1A}$ receptors were determined at human cloned receptors, monitoring the production of cAMP in the presence of forskolin with a concentration up to 10 μm of each ligand [17].

Syntheses of Ligands and Precursors for Labeling

*(N-(2-(1-(4-(2-Methoxyphenyl)piperazinyl)ethyl))-N-pyridinyl)cyclohexane-1′-methyl carboxamide (SWAY) (**3**).* A mixture of amine **5** (914 mg; 2.93 mmol), α-methylcyclohexanecarbonyl chloride (565 mg; 3.51 mmol), triethylamine (0.61 ml; 444 mg; 4.39 mmol) and a catalytic amount of 2-(dimethylamino)-pyridine in dichloromethane (100 ml) was stirred at room temperature (RT) for 19 hours. The reaction was quenched by addition of sodium carbonate solution (10% w/v). The organic layer was separated and the aqueous layer extracted with dichloromethane. The organic layers were dried over magnesium sulfate, filtered, and concentrated. The product **3** was obtained as a colorless oil (638 mg; 50%) after separation on silica gel eluted with dichloromethane–methanol (95:5 v/v). TLC (silica gel, CH_2Cl_2– MeOH, 10:1 v/v). IR (neat): 1640, 1584, 1498, 1465 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.44 (dd, J = 5 Hz, J = 2 Hz, 1H); 7.69 (t, J = 7 Hz, 1H); 7.32 (d, J = 8 Hz, 1H); 7.21–7.17 (m, 1H); 6.95–6.78 (m, 4H); 3.83 (t, J = 7 Hz, 2H); 3.79 (s, 3H); 2.99 (m, 4H); 2.64–1.20 (m, 8H), 1.13 (s, 3H); 0.96–0.87 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.05, 154.45, 149.75, 146.30, 138.85, 135.65, 120.35, 120.20, 118.40, 115.60, 108.70, 53.30, 52.85, 51.05, 48.15, 46.40, 43.10, 34.80, 24.95, 23.40, 21.00. MS (ES): m/z 437 $[M + H]^+$.

*(N-(2-(1-(4-(2-Hydroxyphenyl)piperazinyl)ethyl))-N-pyridinyl)cyclohexane-1′-methyl carboxamide (**6**).* To compound **3** (0.39 g; 0.89 mmol) dissolved in benzene (5 ml) was added aluminum chloride (0.72 g; 5.37 mmol). The reaction mixture was refluxed under nitrogen for 23 hours. The mixture was quenched with water plus sodium carbonate solution (10% w/v) and extracted with ethyl acetate. Combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated. Column chromatography on silica gel (CH_2Cl_2–MeOH; 98:2 v/v) provided the desired product **6** as a colorless oil (295 mg; 78%). TLC (silica gel; CH_2Cl_2–MeOH: 9:1 v/v). IR (neat): 3342, 1639, 1583, 1494, 1464 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.50 (dd, J = 5 Hz, J = 2 Hz, 1H); 7.76 (dt, J = 8 Hz, J = 2 Hz, 1H); 7.36–6.80 (m, 6H); 3.89 (t, J = 8 Hz, 2H); 2.87–2.72 (m, 4H); 2.67 (m, 6H); 1.62–1.24 (m, 8H); 1.19 (s, 3H); 1.14–0.88 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.10, 155.35, 149.95, 147.40, 137.45, 136.70, 124.90, 121.25, 121.05, 119.85, 118.45, 112.50, 54.20, 52.50, 51.05, 47.40, 44.10, 35.75, 25.95, 24.40, 22.00. MS (ES): m/z 423 $[M + H]^+$.

*1-(2-Chloro-propionyl)-4-(2-methoxyphenyl)piperazine (**8**).* 2-Methoxyphenyl piperazine **7** (13.2 g; 68 mmol), anhydrous tetrahydrofuran (THF) (100 ml), and triethylamine (11.5 ml; 85 mmol) were added to a round-bottom flask with a magnetic stirrer under nitrogen. α-Chloro-propionyl chloride (8.7 g; 68 mmol) was then added dropwise with stirring. The reaction mixture was stirred at RT overnight, diluted in ethyl acetate and then washed successively with 1.0 M HCl, $NaHCO_3$ solution (10% w/v) and

[2] *P* is the *n*-octanol-water partition coefficient for a neutral compound. D is the distribution coefficient for all charged and neutral species of the same compound between octanol and buffer at pH 7.4.

water. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give **8** as a yellow oil (16.1 g; 83%). ^{1}H NMR (200 MHz; CD_3CN): δ 7.01–6.90 (m, 4H); 4.84 (q, J = 6.6 Hz, 1H); 3.83 (s, 3H); 3.66 (m, 4H); 3.01 (m, 4H); 1.58 (d, J = 6.6 Hz, 3H). ^{13}C NMR (62.9 MHz; CD_3CN): δ 168.2 [C]; 153.4 [C]; 142.0 [CH]; 124.2 [CH]; 121.9 [CH]; 119.4 [CH]; 112.8 [CH]; 56.0 [CH_3]; 50.7 [CH]; 51.4 [CH_2]; 46.9 [CH_2]; 43.2 [CH_2]; 21.5 [CH_3]. MS: m/z 283/285 [M + H]$^+$. HRMS [M + H]$^+$: 283.1204 (calculated for $C_{14}H_{19}N_2O_2Cl$: 283.1213).

*(1-(2-Methoxyphenyl)-4-[(1-keto)-(2-methyl)-(2-(2-aminopyridinyl)ethyl))piperazine (**9**)*. 2-Aminopyridine (2.6 g; 28 mmol) was stirred with potassium-*t*-butoxide (3.556 g; 31 mmol) under nitrogen in anhydrous *N*,*N*-dimethylformamide (DMF) (100 ml). The amide **8** (8.0 g; 28 mmol) was dissolved in anhydrous DMF and then added portionwise. The reaction mixture was stirred at RT overnight, dissolved in ethyl acetate, and washed with water. The organic layer was separated, dried over magnesium sulfate, and concentrated under reduced pressure to give a yellow oil, which was chromatographed on silica gel (ether–methanol, 15:5 v/v) to give the product **9** as a pale yellow oil (8.5 g; 89%). ^{1}H NMR (200 MHz; CD_3CN): δ 8.01 (d, 1H); 7.37 (m, 1H); 7.00–6.89 (m, 4H); 6.56–6.51 (m, 2H); 5.65 (d, J = 8.0 Hz 1H); 5.01 (m, 1H); 3.82 (s, 3H); 3.69 (m, 4H, 2.99); 1.32 (d, J = 6.9 Hz, 3H). ^{13}C NMR (62.9 MHz; CD_3CN): δ 173.2 [C]; 159.0 [C]; 153.4 [C]; 148.6 [CH]; 142.1 [C]; 138.0 [CH]; 124.1 [CH]; 121.9 [CH]; 119.3 [CH] 113.7 [CH]; 112.7 [CH]; 109.8 [CH]; 56.0 [CH_3]; 47.1 [CH]; 51.6 [CH_2]; 51.2 [CH_2]; 46.4 [CH_2]; 43.0 [CH_2]; 18.8 [CH_3]. MS: m/z 341 [M + 1]$^+$. HRMS: 341.1966 [M + H]$^+$ (calculated for $C_{19}H_{25}N_4O$, 341.1978).

*1-(2-Methoxyphenyl)-4-((2-methyl)-2-(2-aminopyridinyl) ethyl)piperazine (**10**)*. Compound **9** (2.0 g; 5.89 mmol) was dissolved in anhydrous diethyl ether (100 ml) and stirred under nitrogen at RT for several minutes. Lithium aluminum hydride in diethyl ether (20 ml; 1.0 M) was added portionwise. The reaction mixture was then stirred at RT overnight and then treated with sodium hydroxide solution (10% w/v), water, and diethyl ether and stirred for 1 hour. The reaction mixture was filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate–ethanol, 20:1 v/v) to give the product **10** as a yellow oil (1.5 g; 79%). ^{1}H NMR (200 MHz; CD_3CN): 7.98 (d, 1H); 7.36 (t, 1H); 6.91–6.89 (m, 4H); 6.52–6.42 (m, 2H); 5.14 (d, J = 6.2 Hz, 1H); 4.00 (m, 1H); 3.78 (s, 3H); 2.96 (m, 4H); 2.57 (m, 4H); 2.48 (ABd, $^2J_{HH}$ = 12 Hz, $^3J_{HH}$ = 7.8 Hz, 1H); 2.33 (ABd, $^2J_{HH}$ = 12 Hz, $^3J_{HH}$ = 6.4 Hz, 1H); 1.18 (d, J = 6.9 Hz, 3H). ^{13}C NMR (62.9 MHz; CD_3CN): δ 160.0 [C]; 153.4 [C]; 148.9 [CH]; 143.0 [C]; 137.9 [CH]; 123.5 [CH]; 121.9 [CH]; 119.0 [CH]; 113.1 [CH]; 112.7 [CH]; 108.9 [CH]; 64.7 [CH_2]; 55.9 [CH_3]; 54.6 [CH_2]; 51.4 [CH_2]; 45.0 [CH]; 20.0 [CH_3]. MS: m/z 327 [M + H]$^+$. HRMS: 327.2172 [M + H]$^+$ (calculated for $C_{19}H_{25}N_4O$: 327.2185).

*(N-(2-(1-(4-(2-Methoxyphenyl)piperazinyl)(2-methylethyl)))-N-pyridinyl)cyclohex-anecarboxamide[(R,S)-JWAY] (**4**)*. Compound **10** (1.49 g; 4.56 mmol) and triethylamine (763 µl; 5.48 mmol) was dissolved in anhydrous THF (30 ml) and stirred under nitrogen. Cylohexanecarbonyl chloride (725 µl; 5.48 mmol) was then dissolved in anhydrous THF (20 ml) and added dropwise. The reaction mixture was stirred at RT overnight and then washed with 1.0 M HCl, saturated $NaHCO_3$, and water. The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the product **4** as a light brown oil (1.2 g; 60%). ^{1}H NMR (200 MHz; CD_3CN): δ 8.54 (d, 1H); 7.84 (dt, 1H); 7.47–7.35 (m, 2H); 6.93–6.9 (m, 4H); 5.0 (m, 1H); 3.80 (s, 3H); 3.0 (m, 4H); 2.65 (m, 2H); 2.50 (m, 2H); 2.38 (ABd, $^2J_{HH}$ = 13 Hz, $^3J_{HH}$ = 9 Hz, 1H); 2.25 (ABd, $^2J_{HH}$ = 13 Hz, $^3J_{HH}$ = 6 Hz, 1H); 1.60–1.01 (m, 14H). ^{13}C NMR (62.9 MHz; CD_3CN): δ 176.4 [C]; 154.4 [C]; 153.4 [C]; 150.1 [CH]; 142.7 [C]; 139.3 [CH]; 126.1 [CH]; 124.2 [CH]; 123.5 [CH]; 121.9 [CH]; 119.1 [CH]; 112.8 [CH]; 62.5 [CH_2]; 55.9 [CH_3]; 54.3 [CH_2]; 51.5 [2 × CH_2]; 43.9 [CH]; 30.6 [CH_2]; 30.1 [CH_2]; 26.5 [CH_2]; 26.4 [CH_2]; 26.2 [CH_2]; 21.1 [CH]; 17.7 [CH_3]. MS: m/z 437 [M + H$^+$]. HRMS: found 437.2903 [M + H$^+$] (calculated for $C_{26}H_{37}N_4O$, 437.2917).

Radiosyntheses of Radioligands

*[O-methyl-^{11}C]SWAY (**11**)*. No-carrier-added (NCA) [^{11}C]methyl triflate was prepared using a GE Medical Systems box [18] and dispensed into a vial containing the hydroxy precursor **6** (0.5 mg; 1.18 µmol), 0.5 M NaOH (4 µl), and acetone (400 µl). The radioactive product **11** was purified with reverse phase HPLC on a µ-Bondapak-C18 column (10-µm particle size; 30 × 0.78 cm) eluted with MeOH–0.1 M HCO_2NH_4–Et_3N (60:40:0.3 v/v) at 6.0 ml/min, with eluate monitored sequentially for absorbance at 254 nm and radiation (GM-tube detector). The fraction containing **11** was collected, rotary evaporated to dryness, and taken up in sterile physiological saline.

*Radiosynthesis of [carbonyl-^{11}C](R,S)-JWAY (**12**)*. Precursor **10** (7.5 mg; 23 µmol) was acylated with [^{11}C]cyclohexanecarbonyl chloride, under the same conditions described for the preparation of [*carbonyl*-^{11}C]WAY-100635 [19]. The radioligand **12** was separated under the same HPLC conditions used to separate **11**. The fraction containing **12** was collected, rotary evaporated to dryness, and taken up in sterile physiological saline.

Evaluation of Radioligands in Cynomolgus Monkey with PET

The study was approved by the Animal Ethics Committee of Northern Stockholm (Sweden).

Two male cynomolgus monkeys were used: one weighing 6.6 kg for [*O-methyl*-^{11}C]SWAY experiments and the other 5.5 kg for [*carbonyl*-^{11}C](*R*,*S*)-JWAY experiments. For each PET experiment, the monkey was anesthetized by repeated intramuscular injection of ketamine–xyalazine [Ketalar 1–2 mg/kg/hour, Rompun 1 mg/kg/hour] and then positioned in a Siemens ECAT EXACT HR PET camera [20] (resolution; 3.8 mm full width half maximum) so that the transaxial imaging planes of the head were parallel to the cantomeatal line. An apparatus was used to secure a fixed position of the monkey during the PET measurements [21]. Monkey body temperature was maintained with a thermostatically controlled heating pad. No-carrier-added [*O-methyl*-^{11}C] SWAY [1.30 mCi; 683 Ci/mmol; 1.9 nmol (0.829 µg) carrier] or [*carbonyl*-^{11}C](*R, S*)-JWAY [1.46 mCi; 189 Ci/mmol; 7.72 nmol (3.36 µg) carrier] was injected into the left sural vein and regional

cerebral radioactivity uptake was measured in 3-D mode for up to 90 minutes and corrected for physical decay. Data were displayed as 47 sections with a separation of 3.3 minutes. Brain regions of interest (ROI) were drawn in the PET summation images, which represented radioactivity measured from nine minutes after injection to the end of scan. ROI and the whole brain contour were defined *in situ* according to an atlas of cryosected cynomolgus monkey head. Radioactivity concentration was calculated from the sequence of time frames, corrected for physical decay, and plotted vs. time. To calculate the percentage of injected radioligand in whole brain, the radioactivity concentration in the ROI for the whole brain was multiplied by the brain volume (estimated to be about 65 ml). The calculated value for radioactivity in the brain, as percentage of injected dose, was then divided by the radioactivity injected and multiplied by 100.

In two separate PET experiments, one of the test radioligands ([*O-methyl*-^{11}C]SWAY at 878 Ci/mmol or [*carbonyl*-^{11}C](*R*, *S*)-JWAY) at 230 Ci/mmol was injected intravenously into the respective monkey, soon (12 minutes) after intravenous injection of WAY-100635 at a dose of 0.5 mg/kg.

Blood Clearance of Radioactivity and Analysis of Radioactive Metabolites in Plasma

The analysis of radioactive compounds present in plasma was determined by an HPLC method that has been shown to be effective for several other PET radioligands [22, 23]. After intravenous injection of radioligand into monkey, a total of four arterial blood samples were taken (at times selected from four, 12, 15, 30, and 45 or 60 minutes) during the scan. Each blood sample was counted for radioactivity and centrifuged at 2000 × *g* for 1 minute. The supernatant plasma (0.5 ml) was deproteinized with acetonitrile (0.7 ml) that had been prespiked with reference ligand. The radioactivity of this mixture was measured in a well counter and a portion (1 ml) was analyzed by injection onto a gradient HPLC system, a μ-Bondapak-C18 column (300 × 7.8 mm o.d.; 10-μm particle size) eluted at 6.0 ml/min with acetonitrile–0.01 M phosphoric acid. More than 98% of the radioactivity in the blood sample was recovered in the deproteinized plasma taken for analysis.

Results

Lipophilicity and Pharmacology

The rank order of calculated log *P* values was (*R*, *S*)-JWAY > CPC-222 > SWAY > WAY-100635, and of calculated log *D* values (*R*, *S*)-JWAY > WAY-100635 > CPC-222 > SWAY (Table 1). SWAY and (*R*, *S*)-JWAY were each found to be high-affinity antagonists at 5-HT$_{1A}$ receptors (Table 1).

Syntheses and Radiochemistry

SWAY and (*R*, *S*)-JWAY were each obtained in moderate overall yield in four steps from commercially available 2-methoxyphenyl piperazine **7**. SWAY was demethylated to precursor **6** in high yield (Fig. 2), and precursor **10** was obtained on the path to (*R*,*S*)-JWAY (Fig. 3). ^{11}C-labeled SWAY and (*R*,*S*)-JWAY were obtained in greater than 99% radiochemical purity, and in 50 and 98% decay-corrected radiochemical yields, respectively (from labeling agent) (Fig. 4). The specific radioactivity of [*O-methyl*-^{11}C]SWAY at the time of injection in the baseline experiment was 683 Ci/mmol and in the pretreatment experiment 878 Ci/mmol. The specific radioactivity of [*carbonyl*-^{11}C](*R*,*S*)-JWAY at the time of injection in the baseline experiment was 189 Ci/mmol and in the pretreatment experiment 230 Ci/mmol.

PET Experiments in Cynomolgus Monkey

[O-methyl-^{11}C]SWAY. In the baseline experiment, radioactivity uptake in whole brain reached a maximum of 3% of the injected dose at 4.5 minutes and decreased to 0.68% at 72 minutes (Fig. 5). A similar pattern of uptake and

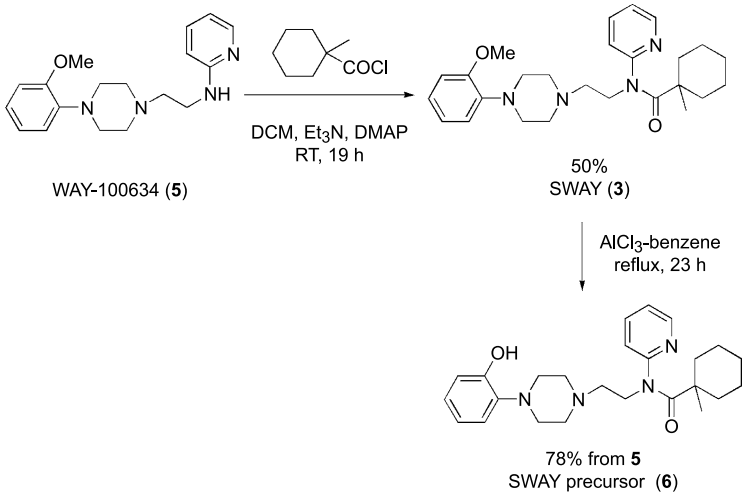

Fig. 2. Synthesis of SWAY and precursor.

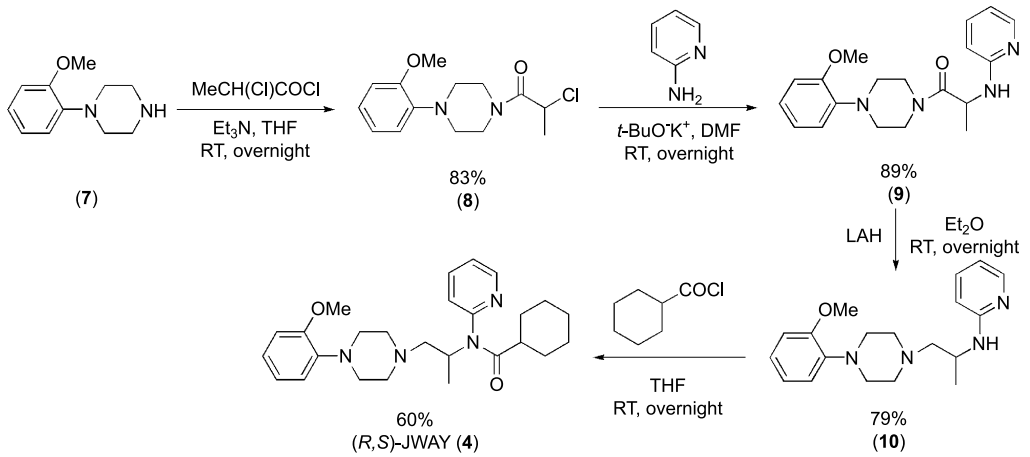

Fig. 3. Synthesis of (*R*, *S*)-JWAY and precursor.

radioactivity clearance was seen throughout the brain, such that no clear PET image of 5-HT$_{1A}$ receptor distribution was obtained. Throughout the 72 minutes of data acquisition, the radioactivity concentrations in all sampled brain regions, including those densely populated with 5-HT$_{1A}$ receptors (e.g., cingulate cortex, frontal cortex, hippocampus, and temporal cortex), were only slightly higher than that in receptor-devoid cerebellum (Fig. 6). Thus, at 72 minutes, ratios of radioactivity in cingulate cortex, frontal cortex, hippocampus, and temporal cortex to that in cerebellum were 1.15, 1.10, 1.10, and 1.16, respectively (Fig. 7). No great effect of pretreatment with WAY-100635 was discernible (data not shown).

[carbonyl-^{11}C](R,S-JWAY). In the baseline experiment, radioactivity uptake in whole brain reached a maximum of 4.8% of the injected dose at 2.5 minutes and decreased to 1.2% of the injected dose at 90 minutes (Fig. 5). At 2.5 minutes, the radioactivity concentration was similar in all regions. Clearance of radioactivity was more rapid from cerebellum than from 5-HT$_{1A}$ receptor-rich regions (e.g., cingulate cortex, frontal cortex, hippocampus, and temporal cortex) (Fig. 8A). A PET image from the summed data collected after nine minutes from injection shows radioactivity distributed primarily according to the distribution of 5-HT$_{1A}$ receptors (Fig. 9A). At 90 minutes, the ratios of radioactivity in cingulate cortex, hippocampus, frontal cortex, and temporal cortex (5-HT$_{1A}$ receptor-rich regions) to that in cerebellum were 2.60, 2.58, 2.20, and 2.15, respectively, and still increasing (Fig. 10A). The corresponding ratios for amygdala, parietal cortex, occipital cortex, caudate nucleus, pallidum, mesencephalon, thalamus, pons, putamen, and hypothalamus were 2.39, 2.05, 1.91, 1.80, 1.39, 1.39, 1.34, 1.33, 1.27, and 1.17, respectively.

In the pretreatment experiment, WAY-100635 reduced retention of radioactivity in 5-HT$_{1A}$ receptor-rich regions to almost the same level as in receptor-devoid cerebellum over the 90 minutes scan (Fig. 8B). Hence, no image of the distribution of brain 5-HT$_{1A}$ receptors was obtained (Fig. 9B). Ratios of radioactivity in hippocampus, temporal cortex, cingulate cortex, and frontal cortex and to that in cerebellum were reduced to 1.42, 1.27, 1.22, and 1.07, re-

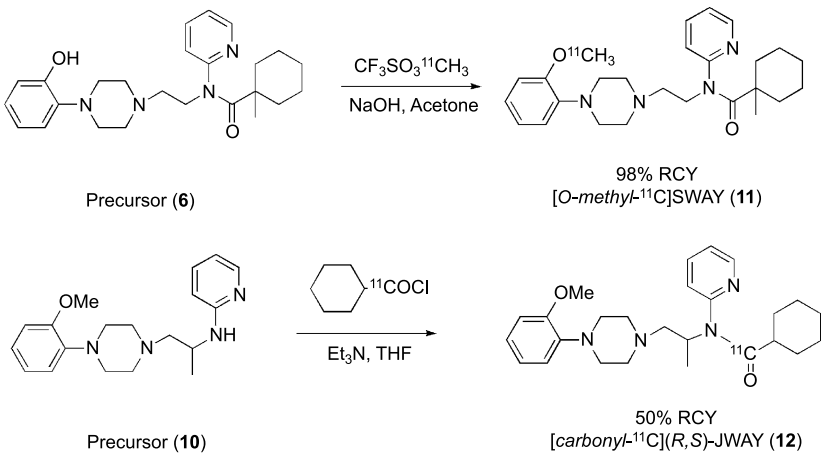

Fig. 4. Radiosynthesis of [^{11}C]SWAY and [^{11}C](*R*,*S*)-JWAY.

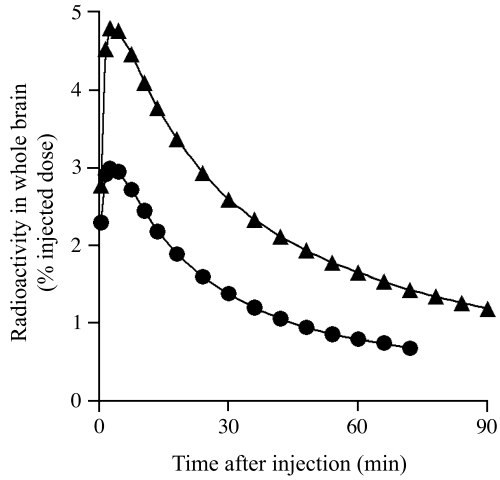

Fig. 5. Whole brain uptake and clearance of radioactivity after intravenous injection of cynomolgus monkey with [^{11}C]SWAY (●) or [^{11}C](*R*,*S*)-JWAY (▲).

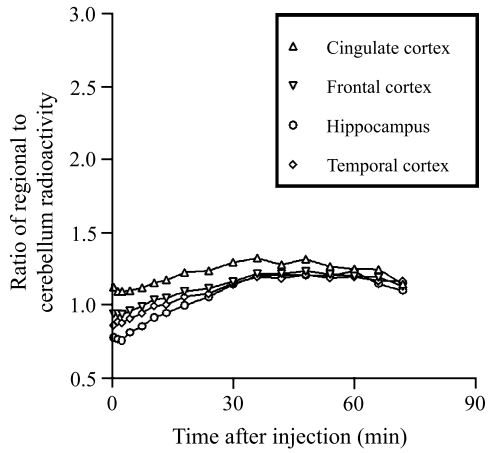

Fig. 7. Ratio of radioactivity concentration in selected brain regions (cingulate cortex, frontal cortex, hippocampus, temporal cortex) to that in cerebellum after intravenous injection of cynomolgus monkey with [^{11}C]SWAY.

spectively (Fig. 10B). The corresponding ratios for amygdala, putamen, caudate nucleus, occipital cortex, thalamus, parietal cortex, hypothalamus, mesencephalon, pallidum, and pons were 1.37, 1.12, 1.08, 1.08, 1.04, 1.03, 0.97, 0.92, 0.85, and 0.74, respectively.

Analysis of Parent Radioligand and Radioactive Metabolites in Monkey Plasma

[O-methyl-^{11}C]SWAY. Radioactivity cleared rapidly from blood ($t_{1/2}$ ~ 5.5 minutes). [*O-methyl*-^{11}C]SWAY was metabolized quite rapidly. Thus, in the baseline experiment, [*O-methyl*-^{11}C]SWAY represented 47% of the radioactivity in plasma at 45 minutes after intravenous injection. In the pretreatment experiment, the corresponding value was 27% (Fig. 11). In the HPLC analysis, radioactive metabolites eluted at 3.2 and 4.0 minutes and were each more polar than the parent radioligand (retention time 6.02 minutes).

[carbonyl-^{11}C](R,S)-JWAY. Radioactivity cleared rapidly from blood ($t_{1/2}$ ~ 7.8 minutes). [*carbonyl*-^{11}C](*R*,*S*)JWAY was rapidly metabolized (Fig. 11). In both the baseline and pretreatment experiments, parent radioligand represented about 12% of the radioactivity in plasma at 45 minutes, respectively. Two radioactive metabolites were observed in the HPLC analysis, one minor (retention time 3.94 minutes) and one major (retention time 2.58 minutes), each more polar than parent radioligand (retention time 5.55 minutes).

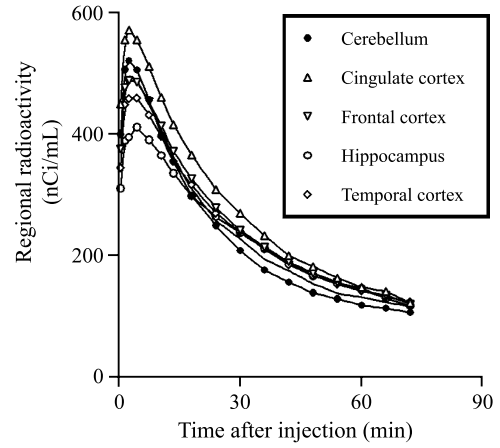

Fig. 6. Uptake and clearance of radioactivity in selected brain regions (cerebellum cingulate cortex, frontal cortex, hippocampus, temporal cortex) after intravenous injection of cynomolgus monkey with [^{11}C]SWAY.

Discussion

Lipophilicity and Pharmacology

Calculated log P and log D values for ligands **1**–**4** fall within a narrow range of 0.53 and 0.39 units, respectively. Since these calculations are of unknown accuracy, it is not easy to state the true rank order of lipophilicities with confidence.

SWAY and (*R*,*S*)-JWAY were found to be antagonists of the 5-HT$_{1A}$ receptor (Table 1). The *R*-enantiomer of JWAY is known to have almost the same low IC$_{50}$ value for the 5-HT$_{1A}$ receptor as WAY-100635, and the *S*-enantiomer a 75-fold higher value [24, 25]. The K_I value determined for (*R*,*S*)-JWAY (0.91 nM) is in accord with the IC$_{50}$ values reported for each of its enantiomers. SWAY was found to have about a sixfold higher IC$_{50}$ value than WAY-100635.

Synthesis and Radiochemistry

The synthesis of SWAY was readily accomplished by acylation of the amine **5**. Reflux of **3** with aluminum chloride in benzene gave the required precursor **6** in high yield. The synthesis of JWAY described here differs from former

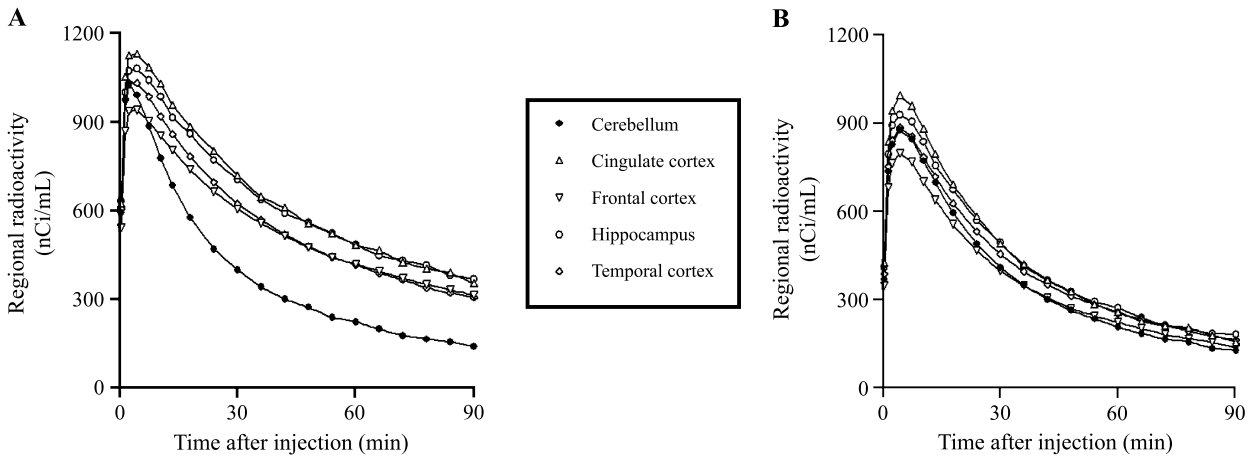

Fig. 8. Uptake and clearance of radioactivity in selected brain regions (cerebellum cingulate cortex, frontal cortex, hippocampus, temporal cortex) after intravenous injection of [^{11}C](*R*,*S*)-JWAY in baseline experiment **(A)** and pretreatment experiment **(B)**.

syntheses [26, 27] and is overall higher yielding. Radiochemical yields of purified [*O-methyl*-^{11}C]SWAY and [*carbonyl*-^{11}C](*R*,*S*)-JWAY were high using ^{11}C methylation- and ^{11}C carboxylation-labeling reactions, respectively. Both radioligands were obtained in moderately high specific radioactivities.

PET Experiment in Monkey Using [O-methyl-^{11}C]SWAY

After injection of [*O-methyl*-^{11}C]SWAY into monkey, there was appreciable penetration of radioactivity into brain. The maximal whole brain uptake of radioactivity was 3% of injected dose at 4.5 minutes (Fig. 5), a value that is substantially lower than those of effective PET radioligands for brain 5-HT$_{1A}$ receptors such as [*carbonyl*-^{11}C]WAY-100635 [28] and [*carbonyl*-^{11}C]desmethyl-WAY-100635 [29]. WAY-100635 and a close structural analog *p*-MPPF, in which the cyclohexyl ring of WAY-100635 is replaced with a *p*-fluorophenyl group, are each known to be substrates of the brain Pgp transporter in rats [30, 31]. Pgp transports WAY-100635 and *p*-MPPF out of the brain.[3] A similar mechanism may act on SWAY to limit brain uptake, although other factors, such as lipophilicity and binding to blood proteins, may also be important.

Radioactivity uptake from all brain regions, including receptor-devoid cerebellum and 5-HT$_{1A}$ receptor-rich regions (e.g., hippocampus, cingulate cortex, frontal cortex and temporal cortex), were quite similar as were clearances of radioactivity (Fig. 6). After 72 minutes, radioactivities in the 5-HT$_{1A}$ receptor-rich regions were only marginally greater than in cerebellum, indicating that only a small fraction of total radioactivity was specifically bound to 5-HT$_{1A}$ receptors (Fig. 7). In view of the very small signal, the effect of pretreatment with WAY-1000635 was difficult to discern and is not reported in detail. The primary reason for the very low receptor-specific signal is probably the sixfold lower affinity of [^{11}C]SWAY compared to WAY-100635.

PET Experiment in Monkey Using [carbonyl-^{11}C](R,S)-JWAY

[*Carbonyl*-^{11}C](*R*,*S*)-JWAY readily entered brain. Maximal whole brain radioactivity uptake was 4.8% of injected dose at 2.5 minutes and comparable to that of other effective PET radioligands in monkey (Fig. 5) [2, 28, 29, 32]. Uptake was similar in all brain regions, but clearance was substantially faster from receptor-devoid cerebellum than from 5-HT$_{1A}$ receptor-rich regions such as cingulate cortex, frontal cortex, hippocampus, and temporal cortex (Fig. 8A). Summed PET data from nine minutes after injection revealed the radioactivity in brain to be mainly distributed according to the known distribution of 5-HT$_{1A}$ receptors (Fig. 9A). Ratios of radioactivity in 5-HT$_{1A}$ receptor-rich regions to that in cerebellum, taken to represent ratios of receptor-specific to nonspecific binding, increased over time. The highest ratio was recorded in cingulate cortex (2.60 at 90 minutes) (Fig. 10). The ratio in frontal cortex was 2.2.[4] By contrast, in a similar study using [*carbonyl*-^{11}C]WAY-100635, the same ratio reached a much higher value (8.5) [7]. An almost twofold higher receptor-specific signal would be expected

[3]The effect is much greater for p-MPPF than WAY-100635.

[4] The specific radioactivity of the radioligand for baseline and pretreatment experiments was not as high as that previously achieved with [*carbonyl*-^{11}C]WAY-100635 [27]. In these experiments, the greater presence of carrier (*R*,*S*)-JWAY may have caused some saturation of receptors, which in turn may have reduced the specific binding of the radioligand.

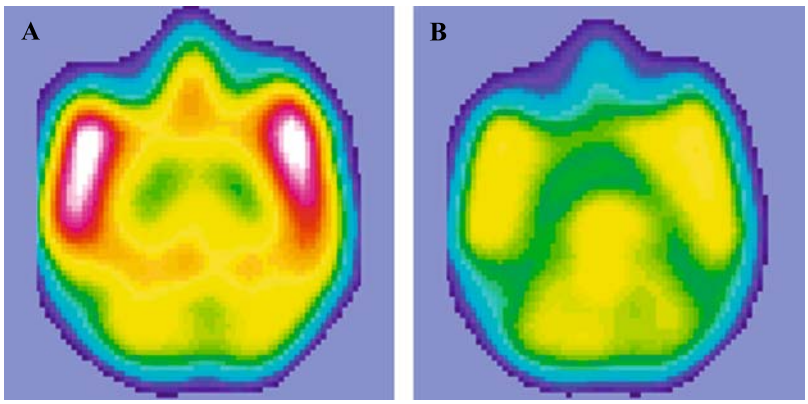

Fig. 9. Transaxial scans of cynomolgus monkey brain after intravenous injection of [^{11}C](*R*,*S*)-JWAY in baseline **(A)** and pretreatment experiment **(B)**.

from the future use of the homochiral and higher-affinity radioligand, [*carbonyl*-^{11}C](*R*)-JWAY. However, even allowing for this gain, the homochiral radioligand would not provide a greatly superior ratio compared to that from the currently most widely used PET radioligand for brain 5-HT_{1A} receptors, [*carbonyl*-^{11}C]WAY-100635.

The receptor-specific signals were almost completely blocked by pretreatment of the monkey with WAY-100635 as shown in the regional time–activity curves (Fig. 8B) and in the ratios of radioactivity to that in cerebellum for different brain regions (Fig. 10B). The PET image of brain 5-HT_{1A} receptors was obliterated by pretreatment with WAY-100635 (Fig. 9B), thereby confirming the 5-HT_{1A} receptor selectivity of the radioligand.

Metabolism of [O-methyl-^{11}C]SWAY

The lipophilicities of the radioactive metabolites of [*O-methyl*-^{11}C]SWAY, as indicated by their retention times on reverse phase HPLC, are consistent with amide hydrolysis as the primary route of metabolism (as known for WAY-100635 and several of its congeners) [2, 33]. [*O-methyl*-^{11}C]SWAY was metabolized at a rate similar to [*O-methyl*-^{11}C]WAY-100635 [23]. Thus, [*O-methyl*-^{11}C]SWAY represented 89 and 39% of the radioactivity in plasma at four and 60 minutes, respectively (Fig. 11). By comparison, in a similar previous study of [*O-methyl*-^{11}C]-WAY-100635 injected into cynomolgus monkey, parent radioligand represented 97 and 49% of the radioactivity at 3 and 59 minutes, respectively. Thus, the presence of the α-methyl group in [*O-methyl*-^{11}C]SWAY has no appreciable influence on rate of metabolism. This accords with findings for the *in vitro* acid or base-catalyzed hydrolysis of amides having an α-methyl group on the carbonyl side of the amide bond [34, 35]. The rapid metabolism of this radioligand may give one or more radioactive species that may enter the brain. Especially, for metabolism by amide hydrolysis, a significant radioactive metabolite will be the amine [*O-methyl*-^{11}C]WAY-100634, which is well known to enter cynomolgus monkey brain [33]. Metabolism may be a

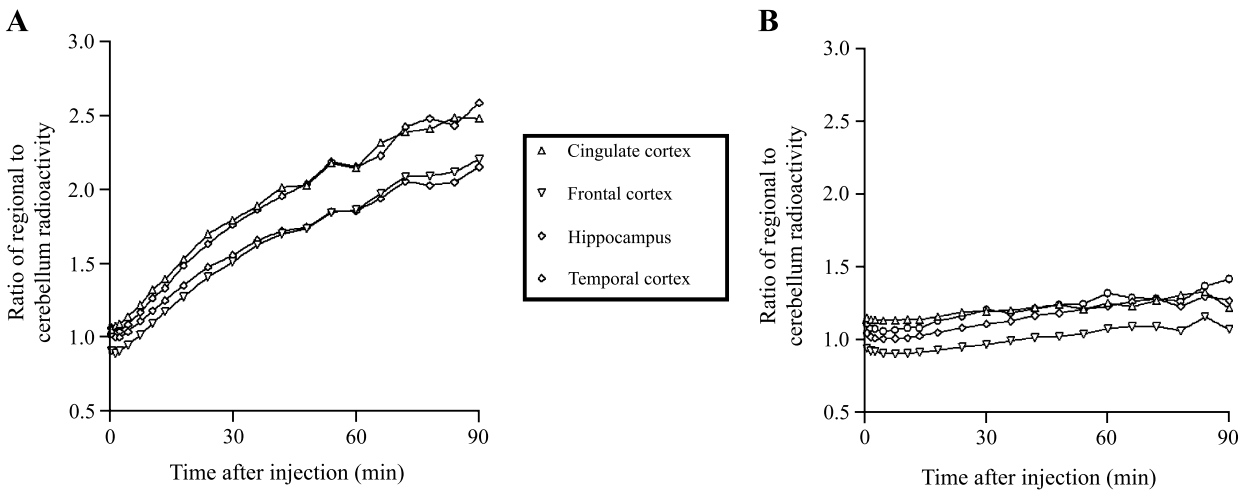

Fig. 10. Ratio of radioactivity concentration in selected brain regions (cingulate cortex, hippocampus, frontal cortex, temporal cortex) to that in cerebellum after injection of cynomolgus monkey with [^{11}C](*R*,*S*)-JWAY in baseline experiment **(A)** and pretreatment experiment **(B)**.

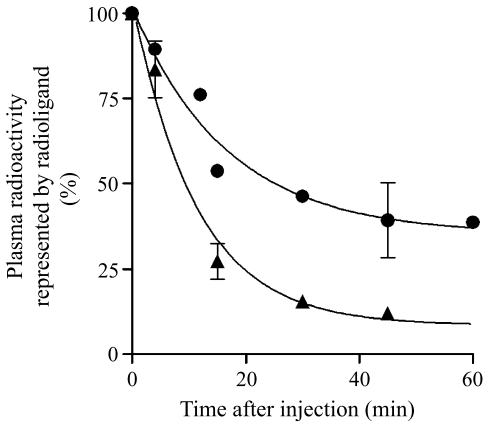

Fig. 11. Percentage of radioactivity in plasma represented by parent radioligand after injection of cynomolgus monkey with [^{11}C]SWAY (●) or [^{11}C](*R*,*S*)-JWAY (▲). For each radioligand, data are averaged from the baseline and pretreatment experiments (error bars show the range of the two values). Curves are derived from monoexponential fits.

further reason for the failure of this radioligand to give a 5-HT$_{1A}$ receptor-specific PET signal in monkey brain.

Metabolism of [carbonyl-^{11}C](R,S)-JWAY

The radioactive metabolites of [*carbonyl*-^{11}C](*R*,*S*)-JWAY have shorter retention times compared to parent radioligand on reverse phase HPLC. This is consistent with amide hydrolysis of the radioligand as the primary route of metabolism. Various reports have shown that the rate of enzymatic hydrolysis of amide bonds (by amidases) in certain substrates is retarded by steric crowding of the bond, even by small groups such as a methyl group in α-position to the amido nitrogen [36–38]. However, here, the introduction of a methyl group on the ethyl chain of WAY-100635 at the α-carbon position to the carbonyl group was found to have no appreciable effect on metabolism in monkey. Thus, after 4 minutes, the radioactivity in plasma representing parent radioligand was 69%, which decreased to 12% by 45 minutes (Fig. 11). By comparison, [*carbonyl*-^{11}C]WAY-100635 similarly represented 69 and 19% of the radioactivity in monkey plasma at the same time points [7].

Conclusions

SWAY and (*R*,*S*)-JWAY were each found to be high-affinity antagonists at 5-HT$_{1A}$ receptors. [^{11}C](*R*,*S*)-JWAY, but not [^{11}C]SWAY, gives a sizeable selective PET signal for 5-HT$_{1A}$ receptors in monkey brain *in vivo*. [*O-methyl*-^{11}C]SWAY is less rapidly metabolized than [*carbonyl*-^{11}C](*R*,*S*)-JWAY, as assessed by a slower appearance of radioactive metabolites in plasma. However, neither the α-methyl group in [*O-methyl*-^{11}C]SWAY nor that in [*carbonyl*-^{11}C](*R*,*S*)-JWAY has an appreciable influence on rate of metabolism in monkey when compared to [*O-methyl*-^{11}C]WAY and [*carbonyl*-^{11}C](*R*,*S*)-JWAY, respectively.

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